Wildlife disease. Recent introduction of a chytrid fungus endangers Western Palearctic salamanders.

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Recent introduction of a chytrid fungus endangers Western Palearctic salamanders

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Abstract: Emerging infectious diseases are reducing biodiversity on a global scale. Recently, the emergence of the chytrid fungus *Batrachochytrium salamandrivorans* resulted in rapid declines in populations of European fire salamanders. Here we screened over 5,000 amphibians from across four continents, and combined experimental assessment of pathogenicity with phylogenetic methods to estimate the threat that this infection poses to amphibian diversity. Results show that *B. salamandrivorans* is restricted to, but highly pathogenic for salamanders and newts (Urodela). The pathogen likely originated and remained in co-existence with a clade of salamander hosts for millions of years in Asia. Due to globalization and lack of biosecurity, it has recently been vectored into naïve European amphibian populations, where it is currently causing biodiversity loss.

One Sentence Summary: Human mediated pathogen dispersal rapidly endangers a vertebrate order.

Main Text: Emerging infectious diseases play a significant role in the ongoing sixth mass extinction (*1*). Fungi comprise a greater threat relative to other taxonomic classes of pathogens and have recently caused some of the most severe die-offs and extinctions among a wide range of organisms (*2*). The classical cause of amphibian chytridiomycosis (*Batrachochytrium dendrobatidis)* has resulted in remarkable disease and declines in a wide variety of amphibian species across the three orders [i.e. frogs and toads (Anura), salamanders and newts (Urodela) and caecilians (Gymnophiona)] (*2*). Recently, a second highly pathogenic chytrid fungus (*B. salamandrivorans*) emerged as a novel form of amphibian chytridiomycosis, and extirpated fire salamander populations in northern Europe (*3,4*) in a region where *B. dendrobatidis* is in a state of stable coexistence with the amphibian communities (*5*).

To predict the potential impact of *B. salamandrivorans* on amphibian diversity more broadly, we first estimated its host range by experimentally exposing 35 species from the three amphibian orders (10 anurans, 24 urodelans and one caecilian) to controlled doses of 5,000 zoospores for 24h (*3*) (Table S1). Except for 5 urodelan taxa for which wild caught specimens were used, all other experimental animals were captive bred. With the exception of 4 urodelan taxa, all experimental animals derived from a single source population. After exposure, animals were monitored daily for clinical signs until at least four weeks after exposure. Infection loads were assessed weekly using qPCR on skin swabs (*6*) and histopathology was performed on all specimens that died. Our results show that colonization by *B. salamandrivorans* was limited to Urodela whereas none of the anuran and caecilian species became infected (Fig. 1, squares). Alarmingly, 41 out of 44 of the Western Palearctic salamanders (Salamandridae and Plethodontidae) rapidly died after infection with *B. salamandrivorans*. The propensity of *B. salamandrivorans* to infect these species was confirmed by its ability to successfully invade the skin of several urodelan, but none of the anuran species. This was demonstrated with an immunohistochemical staining of the abdominal skin of amphibians after exposure to 10,000 zoospores for 24h (Table S1, Fig. S1).

To estimate the current range of *B. salamandrivorans* infections, we used qPCR to screen 5,391 wild amphibian individuals from four continents for the presence of its DNA in their skin (*6*) (Table S2, Table S3). In accordance with the results of the experimentally determined host range, infections were detected only in urodeles. Furthermore, the detection of *B. salamandrivorans*' DNA (all sequences were 100% identical with GenBank accession number

KC762295) was limited to East Asia (Thailand, Vietnam and Japan) in the absence of obvious disease, and Europe (The Netherlands and Belgium) where it is associated with severe disease outbreaks [The Netherlands, 2010 (*3*, *4*) and Belgium, 2013 (Eupen, N 50°37'23"; E 6°05'19"), 2014 (Robertville, N 50°27'12"; E 6°06'11")]. These findings suggest long term endemism in Asia and a recent incursion in Europe.

We used the results of our infection experiments as a proxy for classifying amphibians into four categories of response to *B. salamandrivorans*: resistant, tolerant, susceptible and lethal (Fig. 1, squares). Although the limited number of source populations used does not allow to estimate within-species variation, responses to infection were highly consistent within a given population. Lethal responses were observed both in specimens from captive bred (10 of 19 taxa) and wild (2 of 5 taxa) urodelans. Our infection experiments indicated three Asian salamanders (*Cynops pyrrhogaster*, *Cynops cyanurus* and *Paramesotriton deloustali*) as potential reservoirs. Seven specimens of these species were capable of limiting clinical disease, and either persisted with infection for up to at least five months with recurring episodes of clinical disease, or even totally cleared the infection (Table S1, Fig. S2). The combined evidence of natural occurrence and experimental maintenance of *B. salamandrivorans* infections indicates that at least these three species may function as a reservoir in Asia.

To investigate whether these amphibian communities may have constituted a reservoir of infection in the past, we estimated when *B. salamandrivorans* diverged from *B. dendrobatidis* and used present-day patterns of susceptibility to reconstruct amphibian susceptibility through time. Our Bayesian estimates of divergence time with a broad prior calibration range resulted in a mean estimate of 67.3 million years ago (mya) (Fig. S3) and a 95% highest posterior density interval of 115.3 to 30.3 mya, indicating that *B. salamandrivorans* diverged from *B. dendrobatidis* in the Late Cretaceous or early Paleogene (Fig. 1, grey bar). Maximum Parsimony and Maximum Likelihood ancestral reconstructions (Fig. 1) of amphibian susceptibility suggest that the potential of being a reservoir evolved in the ancestors of modern Asian newts between 55 and 34 mya in the Paleocene (Fig. 1, orange branch), shortly after the origin of their pathogen. These ancestors reached Asia after withdrawal of the Turgai Sea (*7*), suggesting that Asia has been a natural reservoir for *B. salamandrivorans* for the past 30 million years. Our detection of *B. salamandrivorans* in an over 150 year old museum sample of the Asian newt *Cynops ensicauda* (Table S4, RMNH RENA 47344) is consistent with this reservoir hypothesis.

Given the discontinuity of the global distribution of *B. salamandrivorans*, introduction from Asia into Europe must have been human-mediated. Asian salamanders and newts are being traded internationally in large numbers annually (for instance, more than 2.3 million individuals of *Cynops orientalis* were imported into the USA during 2001-2009) (*8*). To assess the potential of *B. salamandrivorans* spread by captive amphibians, 1,765 skin samples from amphibians in pet shops in Europe, London Heathrow airport and an exporter in Hong Kong (Table S5, Table S6) and 570 samples from other captive amphibians (Table S7, Table S8) were tested for *B. salamandrivorans*. We found three positive samples from captive individuals of the Asian newt species *Tylototriton vietnamensis*, two of which were imported to Europe in 2010. Furthermore, our transmission experiments showed that *B. salamandrivorans* can effectively be transmitted across multiple urodelan species (e.g. from *Cynops pyrrhogaster* to *Salamandra salamandra*, Fig. S4) by direct contact demonstrating the potential for pathogen spillover.

Our infection experiments show *B. salamandrivorans* is lethal to at least some of the New World salamandrid species (genera *Taricha* and *Notophthalmus*). Although these combined

genera contain only 7 species, together they have a widespread distribution and are often very abundant. The outcome of exposure of three lineages of plethodontids (a family comprising 66% of global urodelan diversity) to *B. salamandrivorans* ranged from a lack of any detectable infection (*Gyrinophilus*), to transient skin invasion (*Plethodon*) and lethal infection (*Hydromantes*), making it likely that other species in this large family are vulnerable.

Our study demonstrates that the process of globalisation with its associated human and animal traffic can rapidly erode ancient barriers to pathogen transmission, allowing the infection of hosts that have not had the opportunity to establish resistance. Thus pathogens, such as those we describe here, have the potential to rapidly pose a threat of extinction.

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All data described in the paper are presented in the Supporting Online Material.

Figure 1. Amphibian susceptibility to *Batrachochytrium salamandrivorans* (Bs) through time. Molecular timescale for 34 species: rectangles indicate the category in which the species were categorized based on the experimental infection tests. Resistant - no infection, no disease; tolerant - infection in the absence of disease; susceptible - infection resulting in clinical disease with possibility of subsequent recovery; lethal - infection resulting in lethal disease in all infected animals. Coloured dots on nodes indicate the results of the Maximum Likelihood ancestral reconstructions ($P > 0.95$). The clade of susceptible Asian salamanders that originated in the early Paleogene is indicated in orange. The 95% highest posterior density for time of divergence between *B. salamandrivorans* and *B. dendrobatidis* is indicated in grey.

Supplementary Materials

Materials and Methods

Figures S1-S5

Table S1-S10

References (9-39)